Claims

What is claimed is:

1. An attenuated *Salmonella* strain comprising a eucaryotic expression vector for the expression of a heterologous gene or heterologous gene fragment or an autologous gene or autologous gene fragment comprised by the vector within an open reading frame wherein the attenuation is suitable for a vaccination of vertebrates.

2. The *Salmonella* strain according to claim 1 wherein the strain is a *S. typhimurium* strain.

3. The *Salmonella* strain according to claim 2 wherein the strain is selected from the group consisting of *S. typhimurium* aroA SL 7207, *S. typhimurium* LT2, and *S. typhimurium* aroA544 (ATCC Accession No. 33275).

4. The *Salmonella* strain according to claim 1 wherein the strain is a *S. typhii* strain.

5. The *Salmonella* strain according to claim 4 wherein the strain is *S. typhii* Ty21a.

6. The *Salmonella* strain according to claim 1 wherein the eucaryotic expression vector is derived from plasmid pCMVβ, wherein the plasmid comprises:

   a) a structural gene of β-galactosidase (β-gal) under the control of a human CMV immediate early promotor,

   b) a splice donor,

   c) two splice acceptor sites between the promotor and the β-galactosidase gene, and facultatively

   d) a polyadenylation site of SV40.

7. The *Salmonella* strain according to claim 1 wherein said heterologous gene or said heterologous gene fragment or said autologous gene or said autologous gene fragment encodes a polypeptide.

8. The *Salmonella* strain according to claim 7 wherein said polypeptide is an immunogenic protein or a protective antigen.

9. The *Salmonella* strain according to claim 1 wherein the heterologous gene is selected from the group consisting of an *Escherichia coli*-β-*galactosidase* gene (lacZ gene), a non-hemolytic truncated variant of a *Listeria monocytogenes*-listerio lysin gene (hly gene), and a truncated variant of a *Listeria monocytogenes*-actA gene (actA gene).

10. A vaccine comprising a *Salmonella* strain according to claim 1.

11. A method for expression-screening of heterologous genomic DNA libraries or heterologous cDNA libraries for the heterologous gene or heterologous gene fragment or autologous gene or autologous gene fragment, transferred by means of the *Salmonella* strain according to claim 1, comprising the steps of:

a) probing RNA for the presence of splice products derived from a splice donor and acceptors of the eucaryotic expression vector by infecting cells *in vitro* with the *Salmonella* strain according to claim 1,

b) extracting the RNA,

c) performing reverse transcriptase polymerase chain reaction of isolated RNA,

d) isolating the splice products by gel electrophoresis, and

e) sequencing the gene or gene fragment.

12. A method for expression-screening of heterologous genomic DNA libraries or heterologous cDNA libraries for the heterologous gene or heterologous gene fragment

or autologous gene or autologous gene fragment, transferred by means of the vaccine according to claim 10, comprising the steps of:

a) probing RNA for the presence of splice products derived from a splice donor and acceptors of the eucaryotic expression vector by infecting cells *in vitro* with the vaccine according to claim 10,

b) extracting the RNA,

c) performing reverse transcriptase polymerase chain reaction of isolated RNA,

d) isolating the splice products by gel electrophoresis, and

e) sequencing the gene or gene fragment.

13. A method of producing an attenuated *Salmonella* strain comprising a eucaryotic expression vector for the expression of a heterologous gene or heterologous gene fragment or an autologous gene or autologous gene fragment comprised by the vector within an open reading frame, wherein the attenuation is suitable for a vaccination of vertebrates, the method comprising the steps of:

a) expressing genetic information from a heterologous DNA library or heterologous cDNA library or an autologous DNA library or autologous cDNA library as a gene or gene fragment by means of a eucaryotic expression vector carried by an attenuated *Salmonella* strain, wherein the attenuation is suitable for a vaccination of vertebrates,

b) vaccinating a vertebrate by oral, nasal, mucosal, intravenous, intraperitonal, intradermal, or subcutaneous administration and gene delivery with the attenuated *Salmonella* strain according to (a),

c) expression-screening heterologous genomic DNA libraries or heterologous

cDNA libraries for the heterologous gene or heterologous gene fragment or autologous gene or autologous gene fragment transferred by means of the attenuated *Salmonella* strain according to (a), and

d) recovering the *Salmonella* strain.

14. A method of producing a vaccine for oral, nasal, mucosal, intravenous, intraperitonal, intradermal, or subcutaneous gene delivery to vertebrates wherein the vaccine comprises an attenuated *Salmonella* strain comprising a eucaryotic expression vector for the expression of a heterologous gene or heterologous gene fragment or an autologous gene or autologous gene fragment comprised by the vector within an open reading frame, wherein the attenuation is suitable for a vaccination of vertebrates, the method comprising the steps of:

a) expressing genetic information from a heterologous DNA library or heterologous cDNA library or an autologous DNA library or autologous cDNA library as a gene or gene fragment by means of a eucaryotic expression vector carried by an attenuated *Salmonella* strain, wherein the attenuation is suitable for a vaccination of vertebrates,

b) vaccinating a vertebrate by oral, nasal, mucosal, intravenous, intraperitonal, intradermal, or subcutaneous administration and gene delivery with the attenuated *Salmonella* strain according to (a),

c) expression-screening heterologous genomic DNA libraries or heterologous cDNA libraries for the heterologous gene or heterologous gene fragment or autologous gene or autologous gene fragment transferred by means of the attenuated *Salmonella* strain according to (a), and

d) recovering the vaccine comprising the *Salmonella* strain.

15. A method of producing a protein as an expression product of an attenuated *Salmonella* strain comprising a eucaryotic expression vector for the expression of a heterologous gene or heterologous gene fragment or an autologous gene or autologous gene fragment comprised by the vector within an open reading frame, wherein the attenuation is suitable for a vaccination of vertebrates, the method comprising the steps of:

a) expressing genetic information from a heterologous DNA library or heterologous cDNA library or an autologous DNA library or autologous cDNA library as a gene or gene fragment by means of a eucaryotic expression vector carried by an attenuated *Salmonella* strain, wherein the attenuation is suitable for a vaccination of vertebrates including humans,

b) vaccinating a vertebrate by oral, nasal, mucosal, intravenous, intraperitonal, intradermal, or subcutaneous administration and gene delivery with the attenuated *Salmonella* strain according to (a),

c) expression-screening heterologous genomic DNA libraries or heterologous cDNA libraries for the heterologous gene or heterologous gene fragment or autologous gene or autologous gene fragment transferred by means of the attenuated *Salmonella* strain according to (a), and

d) recovering the protein.

16. A method of producing an immune response to an attenuated *Salmonella* strain comprising a eucaryotic expression vector for the expression of a heterologous gene or heterologous gene fragment or an autologous gene or autologous gene fragment

comprised by the vector within an open reading frame, wherein the attenuation is suitable for a vaccination of vertebrates, the method comprising the steps of:

a) expressing genetic information from a heterologous DNA library or heterologous cDNA library or an autologous DNA library or autologous cDNA library as a gene or gene fragment by means of a eucaryotic expression vector carried by an attenuated *Salmonella* strain, wherein the attenuation is suitable for a vaccination of vertebrates, and

b) vaccinating a vertebrate by oral, nasal, mucosal, intravenous, intraperitonal, intradermal, or subcutaneous administration and gene delivery with the attenuated *Salmonella* strain according to (a).